Heat shock protein β-6 emerges as a potential biomarker to predict meat tenderness

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ABSTRACT

The mechanisms controlling meat tenderness involve a multitude of cellular functions. A novel approach to identify the protein(s)/peptide(s) bands associated with tenderness, measured by Warner Bratzler Shear (WBS), is the use of functional proteomics. The aim of this study was to associate electrophoretically separated bands from a muscle myofibrillar fraction with WBS and determine the sequence of the protein(s)/peptide(s) within those bands. Samples for functional proteomics and for WBS were taken at 36h and 72h postmortem, respectively from the *Longissimus dorsi* of 22 Angus crossbred steers. Myofibrillar fractions were resolved on 10% to 20% poly-acrylamide gradient gels. Gels were digitized and analyzed. Resulting data were fitted by a stepwise multiple linear regression model which identified two significant bands (R^2 = 0.508). These bands were analyzed by nano-LC/MS/MS. In the first band, myosin heavy chain and myosin light chain 2 were identified. The second band contained multiple isoforms of myosin light chain 2 and the heat shock protein β-6 (hspb6). This protein, hspb6, has never been associated to tenderness variability. Hspb6 has been related, *in vivo*, to muscle relaxation during situations of acute stress and is known to be intimately involved in the stability of the actin thin filament. The band that contained hspb6 was negatively associated with WBS suggesting that higher levels of hspb6 lead to tougher meat while lower levels lead to more tender meat. By identifying the mechanisms through which tenderness is mediated, it will be possible to implement breeding strategies to produce cattle with greater and more consistent tenderness.

INTRODUCTION

- Meat tenderness
  - One of the most important of all the organoleptic characteristics *(1).*
  - Current USDA beef quality grading system is unable to accurately segregate carcasses into tenderness categories *(2, 3).*
  - Inconsistency results in consumers being dissatisfied *(2).*
  - Consumers are willing to pay a premium for beef of known tenderness *(4).*
- Meat tenderness mechanisms
  - Involves a multitude of cellular functions.
  - Functions like: genetics *(5),* final pH *(6),* the cathepsins, the calpain/calpastatin system, the proteasome *(7),* collagen content, collagen cross-linking, myofibrillar degradation *(8),* and more recently, heat shock proteins *(9, 10).*
- Functional proteomics implementation in meat tenderness research
  - Combination of electrophoretic, image, statistical and protein sequencing technologies that identifies the protein(s)/peptide(s) to a variable *(11).*
  - Can be implemented to study tenderness *(12).*

OBJECTIVE

- ***Perform a functional proteomic analysis to associate electrophoretic bands from the myofibrillar fraction of meat at 36 h postmortem that are associated to meat tenderness at 72 h***
- ***Determine the sequence of the protein(s)/peptide(s) contained in those bands.***

METHODS

- Methodology details
  - 22 Angus crossbred steers.
  - Animals were harvested following USDA approved guidelines.
  - Myofibrillar (insoluble) fraction was obtained through a low salt extraction of soluble proteins.
  - A 10 to 20% gradient poly-acrylamide SDS resolving gel was used.
  - Gels were fluorescently stained and laser scanned.
  - A stepwise multiple linear regression was fitted.
  - Swiss-Prot/TrEMBL v55.3 database was used.
  - Sequencing results were manually trimmed.

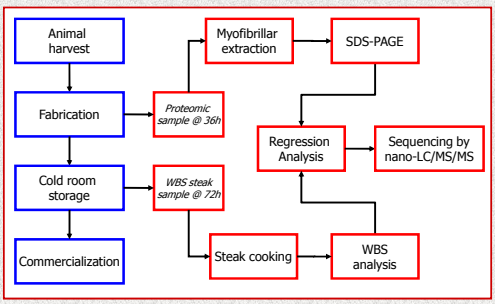

Figure 1. Flowchart of the procedures carried out for this study. Blue boxes correspond to meat industry procedures. Red boxes indicate the present study procedures.

RESULTS

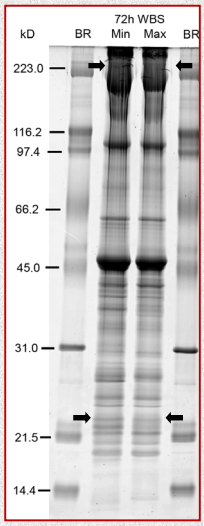

Figure 2. Comparative 10 to 20% gradient SDS-PAGE gel showing the samples that observed the minimum (Min) and the maximum (Max) value for 72 h WBS. The arrows show the location of the bands found to be significantly associated to the dependent variable. BR is the Broad Range molecular weight marker.

Table 1. Descriptive statistics for Warner-Bratzler Shear force (WBS).

Dependent Variable	n	Mean	Std. Dev. (±)	Minimum	Maximum	Range
72 h WBS	22	53.37 N	12.58 N	37.06 N	75.82 N	38.76 N

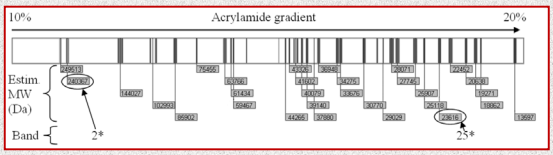

Figure 3. Representation of the synthetic lane used to align and compare the SDS-PAGE profiles. The 30 resolved bands are displayed. Estimated molecular weights (MW) were calculated using the Broad Range standard. The bands found to be associated with WBS are circled.

Table 2. Sequencing data of bands associated with 72 h WBS. The comparison between the pooled sample of animals with the highest WBS values (H) versus the pooled sample of animals with lowest WBS values (L) is displayed.

Band No. (MWe[4] (kDa))	Assoc. Type[1]	Identification	Swiss-Prot Accession No.	MOWSE Scores[2] (H/L)	Fragments Identified[3] (H/L)	MWt[5] (kDa)
2 (240.4)	+	Myosin heavy chain 1	MYH1_BOVIN	5672/6451	272/341	223.76
		Myosin heavy chain 2	MYH2_BOVIN	5647/6100	257/328	224.09
		Myosin heavy chain 7	MYH7_BOVIN	3552/4095	165/196	223.89
		Sarcoplasmic reticulum calcium ATPase 1	AT2A1_BOVIN	229/284	5/7	110.53
		Myosin light chain 2, skeletal	MLRS_BOVIN	0/76	0/3	19.11
25 (23.6)	–	Myosin light chain 2, skeletal	MLRS_BOVIN	929/895	43/37	19.11
		Myosin light chain 2, ventricular	MLRV_BOVIN	580/406	29/16	18.97
		Heat shock protein beta 6	HSPB6_BOVIN	144/146	5/5	17.51
		Creatine kinase M type	KCRM_BOVIN	130/105	5/2	43.19
		Troponin C, slow skeletal & cardiac	TNNC1_BOVIN	120/0	3/0	18.52
		Crystallin alpha B chain	CRYAB_BOVIN	47/0	2/0	20.02

[1] Type of association with the dependent variable; [2] The MOWSE score is a numeric descriptor of the likelihood that the identification is correct; [3] Number of fragments sequenced and matched to that particular protein; [4] MWe is the experimental molecular weight; [5] MWt is the theoretical molecular weight.

SUMMARY

- WBS values were observed to be associated with structural proteins.
- WBS was also associated with a heat shock protein, hspb6.
- The heat shock protein, hspb6, has never been reported before to be associated to meat tenderness.

AKNOWLEDGEMENTS

We would like to thank Dr. H.N. Zerby, Dr. J.M. Reddish at the Department of Animal Sciences of The Ohio State University and the staff and students at the OSU meat lab for their help.

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